The Cellular Mechanisms of Aging

An overview of the molecular genetics and structural biology behind senescence

Dahl Clark
Department of Biology, Duke University, Durham, NC 27708, USA
NSF Graduate Fellowship Recipient now applying to graduate school for fall 2005

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Please send all comments and helpful suggestions to dbc4@duke.edu.

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Introduction

There has been much research done on the genetics of aging, but most of it is unavailable to a nonscientific audience. As a consequence, many people may not be aware that the knowledge and biotechnology exists today to lengthen lifespan in a variety of organisms, including humans.

This paper offers a semi-technical review of what we currently know about the mechanisms behind aging. Aging can be properly defined as a degenerative genetic disease. If left untreated, it will lead to 100% mortality within 122 years of birth (the maximum known human lifespan). It is not a natural process as many of us have come to believe. Instead, this widespread disease is a result of 1) our own genetics, 2) instabilities in our DNA provoked by environmental causes, 3) accumulation of non-recyclable cellular byproducts, and 4) accumulation of cells that have senesced due to a combination

of the above factors. Aging should not go untreated now that we know of many genes that control this process, as well as genes that seem to treat its symptoms.

Today there are non-cancerous cells from many organisms, including humans, that are normal in every respect except that they do not age. Some examples of these otherwise normal, "immortal" cells are included in this paper. They have enjoyed extensions of at least 3 times normal lifespan and may perhaps live even longer. These cells have only simple genetic alterations. They are living proof that it is possible to postpone the aging process via genetic manipulation.

At this moment, researchers have the tools and experience to extend lifespan on a cellular level. Could it be done in whole organisms with current techniques in gene therapy? We don't know because the research has yet to be done. This research in animals and humans has such great promise of extending healthy, useful life that it seems incomprehensible that so few researchers are committed full-time to the genetics of aging. One obstacle could be the lack of enough funding; another could be the lack of a convincing research proposal on how to lengthen lifespan. The conclusion of this paper attempts to present such a proposal, involving current and reliable biotechnology. There is also one last obstacle, the most important to consider.

The Ethics of Extending Lifespan

When a generation becomes the first to do something never before done in nature, there are often many unanswered questions. The foremost of these is "Should we?" Should we build airplanes, because if we were intended to fly we would have been given wings? Should we use our resources to explore Mars so we can learn more about our origins, or improve living conditions on our current planet? Should we genetically alter foods so fewer resources can be used to feed more people in our already overpopulated world, without doing anything else to make their lives more comfortable?

As you are reading this today, the question is once again, "Should we?" With the molecular tools and understanding available for the first time, should we try to extend the useful, disease-free lives of our species? Should we attempt to postpone the "natural" process of dying? What if people live longer but birth rates don't decline—won't the extra pollution and resource use this negatively affect our environment? Will this technology be available to all people or the select few who can pay copiously for it? There will undoubtedly be people who do not think we should tinker with nature, or who believe that trying to postpone death is futile. It should be known that aging is not the norm for all forms of life. For us, it is a degenerative disease that carries 100% mortality, and people with it deserve treatment as with any other serious disease. The technology to treat aging and extend life is here today. What will we do with it?

My Hope

I want to learn to use the tools of genetics and molecular biology so I can eventually work toward extending life in some organism, whether it be a simple eukaryote, mice, or humans. This is why I am applying to graduate school. I may not have worked in a genetics lab but I have done my reading, as I hope to demonstrate with this paper. I know about current research. I know about the tools currently available. Now I would like the opportunity to learn how to use them.

As a human being, I have a choice just as you do. I can live out my life until I die, like every other human being has since the beginning of our species, or I can try to take advantage of today's tools and change things for everyone. Someone once said a person hasn't lived until he or she has found something worth dying for. Well, we will all die anyway, but how many of us have found something worth living for? It is important for me to at least try to do something. Nobody wants to watch helplessly as loved ones deteriorate and die from a treatable disease. However, few people seem willing to work through 5 years of graduate school to learn the science and technology that will enable them to realistically help.

My wish is to see the future, in the company of those I care about and love. I am curious how our species will decide to do things a hundred years from now. Where will our free will and desire to live better take us? How will we have changed? Will we have learned to live in balance with the environment? Will we have learned that wars are not solutions to problems, but the source of problems? It is my curiosity that makes me desire to experience the next day, whether it be a good or bad one. What other creature can experience a day as we can, or work to find ways to ensure more such days can be experienced?

This is a fruitful dream worth passing along to others, a dream of someday being able to exceed our biological programming. Every new bit of research makes that day of freedom draw closer, every voice of support, every eager student who wants to learn how to make this dream a reality.

The Genetics of Aging

What is Senescence?

Senescence is the process by which a cell stops dividing and assumes an altered state of protein production. A cell gradually ages until it reaches senescence. Technically, senescence is an indefinite block to the cell cycle and further replication. The cell cycle usually arrests at the G1-S phase transition, before new DNA synthesis occurs, but can also arrest at the G2-M transition before mitosis.[1] The arrest is a sign that something has gone terribly wrong in the cell so that it cannot replicate, but not wrong enough that apoptosis is triggered.

In vitro, this block to replication can occur in dividing cells by activating either the telomere-dependent (replicative senescence) or telomere-independent (cellular senescence) pathways. Replicative senescence can be halted simply by forcing the expression of the hTERT gene to restore the function of telomerase, an enzyme that maintains the ends of chromosomes. Cellular senescence, however, has a more complex cause resulting from widespread oxidative damage.

In the absence of cell division, cells can still be induced into a senescence-like state by exposure to a fairly wide range of environmental stimuli, such as ectopic expression of RAS-V12 and ceramide treatment.[2]

Regardless of how they are generated, all senescent states involve genome-wide transcriptional changes in the arrested cell, resulting in a dramatically different phenotype from its previous state (Table 1, right). Senescent cells exhibit multiple changes in gene expression, including down-regulation of cell cycle and stress response genes, and up-regulation of matrix-remodeling proteins.[3] This complex phenotype suggests that high-order changes in chromatin structure may be involved in the generation of the senescent phenotype. This altered phenotype also negatively affects the tissue microbalance. Senescent fibroblasts, for example, secrete proteases, anti-angiogenic factors, growth factors, and inflammatory cytokines, all of which are molecules that can be detrimental if not controlled. Thus, the mere presence of senescent cells contributes to the aging of tissue.[4]

Table 1
A selection of genes which display altered transcription with the onset of the senescent state

Repressed at onset of senescence	Upregulated at onset of senescence
c-Fos	Collagenase
Cyclins A and D	Gas 1
IGFI	TIMP-2
TGFβ	PAI 2
Interleukin 6	Fibronectin
Id 1, 2 and 4	Interleukin 1α and β
α1 (III)-Procollagen	Interleukin 15
EPC1	ICAM-1
Microphthalmia-associated transcription factor (Mitf)	MMP3
	MMP10
	IGF-BP2, 3 and 5
	Ws3.10

Replicative and Cellular Senescence

In replicative senescence, the gradual attrition of telomeres eventually leads to enough of a shortening to trigger a p53 and p21waf-mediated cell cycle arrest. The attrition can be caused by end-replication loss alone, or what is more likely in vivo, end-replication loss accompanied by additional telomere loss due to oxidative damage. Evidence for this comes from the replicative immortalization of several different presenescent cell types by forcing them to express hTERT, the catalytic subunit of telomerase.[5]

In cellular senescence, ectopic expression of hTERT alone is not sufficient for immortalization. Instead, p16INK4A, an inhibitor of CDK 4-cyclin D and CDK6-cyclin E kinase pairs which can be upregulated by a wide variety of stimuli including senescence-causing genes, becomes overexpressed, which leads to the hypophosphorylation of retinoblastoma protein pRB.[6]

Interestingly, neither of these two mechanisms appears to be responsible for cellular senescence in rodents. Rodents are short-lived animals that die of cancer instead of old age. A possible cause appears to be a telomere-independent but p53-dependent mechanism caused by the p19arf gene product, possibly resulting from chronic oxidative stress in culture.[7]

Senescence and Tumor Suppression Genes

Senescence is mediated by CDK inhibitors, and controlled by the two most important tumor suppressor genes – p53 and pRB (retinoblastoma gene). p53 is a transcription factor known to mediate a G1 arrest in response to DNA damage. The nuclear protein pRB controls the expression of other genes and halts the cell cycle in response to supermitogenic signals, damage, or oncogenes. Oncogenes act by allowing cells to bypass the senescence response – they give cells a continuous "go" signal to divide regardless of any unrepaired DNA or structural damage that might exist.

All senescent states somehow involve the p16Ink4a/pRb tumor suppression pathway. p16Ink4a is one key link between cellular senescence and cell cycle regulation, and is often mutated or inactivated in normal-appearing and cancerous immortal cells.[8] The p53 gene, if mutated to resemble active p53, gives enhanced tumor suppression but also symptoms of early aging.[9]

Both p53-dependent and p53-independent pathways may exist to control the G1-S cell cycle transition.[10] In human diploid fibroblasts, multiple targets have been identified downstream of p53--p21[11], Gadd45, Mdm2, and cyclin G. p21 has been shown to potently inhibit CDK2,4-cyclin-A,E-kinase activity.[12] Gamma irradiation of human diploid fibroblasts leads to induction of p53 and p21 and a G1 block. Interestingly, these cells show morphological changes reminiscent of cellular senescence[13], and p21 was shown to be upregulated in senescent human fibroblasts.[14]

Comparing Senescence Across Species

Senescence is a universal feature in metazoans. In plants, more than 30 senescence-associated genes have been found, though their senescence is regulated and not passive and time-dependent as in animals.[15] As in animals, senescence in plants is triggered in times of stress, as when exposed to UV-C or pathogens.[16] Fungi appear to be an exception—most fungi are potentially immortal.[17] All natural *Podospora* isolates senesce, but most *Neurospora* isolates are immortal, though 40% of a strain of *Neurospora* in Hawaii carry a senescence plasmid and therefore senesce.

Animals and plants senesce, and some fungi also senesce. How far back in the evolutionary tree might senescence be traceable? If senescence occurs in all eukaryotes, then perhaps there is something special about the eukaryotic condition that has necessitated senescence. There is no senescence in prokaryotes. Being single-celled versus multi-celled also has no bearing over whether an organism has senescence, since yeast are unicellular and still senesce.

The differences between prokaryotes and eukaryotes are many. All eukaryotes have mitochondria, while not all eukaryotes have chloroplasts. We will see later that much of the structural degradation in plant chloroplasts is very similar to the degradation observed in animal mitochondria, which may prove to be an interesting focus of research. Eukaryotes have an end-replication problem with their chromosomes because unlike prokaryotes, they lack circular chromosomes. Finally, eukaryotes also have membrane-bound organelles, which means that cellular ingredients are compartmentalized, and their transportation strongly depends on membrane composition and integrity.

The smallest known eukaryote, a microsporidian called ***Encephalitozoon cuniculi***, is an intracellular parasite of eukaryotic cells.[18] Its genome is less than 3 Mb in length, which is more than 1000 times smaller than the human genome. Its genes are also 20-30% smaller than homologous eukaryotic or bacterial genes. Its genome has been pared down through evolution to contain only those genes necessary for its parasitic lifestyle. It would be interesting to learn whether this tiny fungus also possesses senescence. If so, the mechanism(s) by which it senesces should be very simple to study, compared to organisms with larger genomes.

Genes Governing Cellular Senescence

Cellular senescence is controlled by a highly specific set of genes in a pathway. Turn off any of these genes and the cell will become immortal. This can be done by deleting one of the genes through mutation, or by adding one of its repressors. Cancerous and "normal" immortal cell lines, for example, often have an additional part of a chromosome (6p or 8q in normal immortal cell lines) that likely encodes for a repressor of one of these senescence genes.

The senescence genes all appear to be of dominant inheritance with high levels of homozygosity; otherwise there would be substantial populations of immortal cells within organisms. The repressors of these genes may be turned off early in development by other repressors or cannot function as senescence repressors in low concentration. If chromosome 6p or 8q encodes one or more senescence repressors, this would explain why adding them to a cell line can make it immortal.

Complementation tests were done between 30 different immortal cell lines to identify senescence-related genes.[19] Four complementation groups emerged (Groups A-D), and the chromosomes containing these senescence genes have largely been discovered. The possibility exists that there could be more than one senescence gene on the following chromosomes.

- The Group A gene is on an undiscovered chromosome.
- Chromosome 1q contains the Group C gene.[20]
- Chromosome 7 contains a gene that inhibits the ALT pathway that maintains telomere length; adding it to immortal cells missing it (Group D) kills the ALT pathway.[21]
- Chromosome 4 contains the MORF4 gene at 4q33-34[22]. Adding it to immortal cells missing it (Group B) restores senescence.[23]

There are 6 MORF-related genes.[24] Only MRG15 on chromosome 15 and MRX on chromosome X are expressed; the others are pseudogenes. MRG15 and MRGX may be involved in transcriptional complexes that effect global changes in

gene expression. MORF4 can disrupt these complexes by acting in a dominant negative manner, thereby inducing senescence. MORF4, MRG15, and MRGX are highly conserved in the 3' region of the gene. They all have nuclear localization signals, helix–loop helix and leucine zipper regions, and phosphorylation sites. MRG15 has an additional 5' region that encodes a chromodomain, while MRGX has a unique 5' region.

Chromosome 3 encodes a repressor of telomerase, at least in the human breast carcinoma cell line 21NT.[25] Senescence may be induced through telomere length regulation. Chromosome 3 produces telomerase suppression much like Chromosome 4.[26]

From the above findings and what we have learned from the genetic variations in "normal" immortal cell lines (to be discussed shortly), immortalization can be achieved by either:

- **deleting/turning off** any one of the above senescence genes: MORF4 (chromosome 4), a gene on chromosome 1q, a gene inhibiting the ALT pathway on chromosome 7, and a non-located Group A gene, or

- **adding** a gene or genes on either 6p or 8q; some i(6p) cells later develop i(8q) and +7, while some i(8q) cells later develop i(1q) and a marker chromosome.

Chromosomes 2, 3, 6q, 11, 18, and X have also been implicated in causing senescence in specific immortal cell lines, though the observed senescence could be due to a dosage effect and not a true genetic event.[27]

Human chromosome 7 causes senescence in Group D cell lines, which have unusually long telomeric sequences but no detectable telomerase activity. In the transfected cells, the telomeres were lost or markedly diminished, but were not altered significantly in non-transfected cells. This suggests that telomere length in these Group D cells are maintained through the ALT pathway, and that chromosome 7 may encode an inhibitor of this pathway.[28]

Normal Immortal Cells

The following three cell lines are unique in that they do not senesce and die, but also are not cancerous. In fact, tests show that they have rather normal phenotypes, and require the same growth factors as normal cells. They are currently being used for studies of skin replacement and cancer, but it would be another burgeoning area of research to study them for what they are, normal cells that have genetically circumvented the aging process.

These three cell lines are all human fibroblasts, epithelial cells that are an important component of the skin. As such, they are routinely subjected to some amount of physical and oxidative environmental stress. A single person is likely to have a range of cell damage amounts in their fibroblasts because some areas of skin may be subjected to photoaging, pressure, and rubbing, while others will not. If this range of fibroblasts can be made immortal experimentally by inactivating senescence genes, it may be possible to understand how these senescence genes interact with other genes to bring about the senescent phenotype. Changes in a senescing cell begin well before the cell actually stops dividing, and for this process to be understood, cells at different stages of their replicative lifespan must be studied. DNA microarrays would be useful to give a broad look at how gene expression changes within a cell during the immortalization process.

Making these cells immortal will turn off the senescence mechanism, but not necessarily erase the damage and other effects that aging has had on the cell. It would be interesting to know whether immortalizing cells late in their replicative lifespan predisposes them to cancer.

Individual human cells have naturally reached immortality without becoming cancerous. This raises the possibility that other non-fibroblast cells, and perhaps even entire organisms, may be able to do the same. If replicative immortality is a matter of switching off senescence genes or turning on their repressors by transfection, then what if this were done in a developing zygote of, say, yeast cells, C. elegans, or the fruit fly? Would the organism be able to develop normally with any one or more of its senescence genes switched off? If not, we would know that these genes also play a major role in the development of at least lower eukaryotes. If development proceeds normally, the resulting adult organism would lack a complete senescence pathway in all its cells, and therefore the organism as a whole should exhibit replicative immortality.

This would be indeed interesting, since cells that have reached replicative immortality have shown lifespan extensions of at least 3 times normal lifespan. Things may be different in an organism that has reached replicative immortality, but significant lifespan extension is still a real possibility. However, it is important to note that the organism would likely not reach a complete cellular immortality, as oxidative damage would eventually cause so much irreversible chaos to cell components that senescence would be triggered by alternative pathways. This is assuming that cancer does not develop before cellular senescence is reached. Still, if an organism has replicative immortality, it should exceed its normal lifespan because senescent cells are not changing their microenvironment and accelerating

age-related damage in the process. This is seen in current immortal cell lines, none of which senesce. With this factor contributing to death removed, organisms with replicative immortality should succumb instead to the changes caused by oxidative damage. The onset of cancer and oxidative damage-induced senescence could be postponed by boosting antioxidant and DNA repair systems.

SIK

SIK stands for "spontaneously immortalized keratinocytes." In the original cultures, cells were of normal karyotype and formed colonies with an efficiency of ~3% through 10 passages. At passage 15, after normal strains senesce, the colony forming efficiency of this cell line increased, stabilizing at ~30% by passage 40. Something must have caused this approximately tenfold increase in colony forming efficiency and nearly 3 times extension of replicative lifespan.[29]

During the increase in colony forming efficiency, cells acquired an extra isochromosome 6p, and showed 5-10 fold increases in expression of cell cycle control proteins cyclin A, cyclin B, and p34cdc2. At later passages, cells also showed i(8q) and +7, but the i(6p) and increased cell-cycle protein expression remained. These latter features were probably important for immortalization. Like normal, non-cancerous keratinocytes, the SIK cells were sensitive to growth suppression by TGF-B and tetradecanoylphorbol acetate, and depend on epidermal growth factor (EGF) for progressive growth.

These results indicate that normal, limited lifespan is the dominant inherited trait, regardless of cell type. Therefore, immortal cells must have a recessive gene for that trait. Any of 30 immortal cell lines have been shown to fit into one of 4 possible complementation groups, which suggests that a small number of highly specific genes are involved in senescence. This set of genes must be turned on for a cell to become senescent, but losing any one of the set will render the cell immortal.

Chromosome 11 is implicated in tumor suppression, but when added to these immortal cell lines, nothing happened. The tumor suppressor gene(s) on Chromosome 11 may stop tumors, but are not involved in the aging process. Chromosome 4 induced senescence in a line of cervical carcinoma cells, but not in other groups. It is not known whether these cancer cells may have shared features with complementation group B, for which MORF4 on Chromosome 4 induces senescence.

NIKS

The NIKS cell line has been patented as ATCC CRL-12191 (previously called BC-1-Ep/SL cells).[30] They show a normal karyotype of 46 chromosomes, except with i(8q). Like normal keratinocytes, they need epidermal growth factor (EGF) for serial passage. They can survive without it, but grow poorly.[31] TGF-beta 1 also inhibits growth. At passage 17, only NIKS cells remained in the culture. At passage 31, all cells had i(8q) only. At passage 54, all cells still had i(8q), but some had i(1q) and a marker chromosome. It appears that the expression of extra 8q genes is the cause of their immortality.

NM1

NM1 is a human keratinocyte line that is trisomic for chromosome 8 and also XY as the NIKS cell line.[32] Being trisomic for chromosome 8, it shares the expression of extra 8q genes with the NIKS cell line, and suggests that the expression of extra 8p genes does not affect immortality. These cells differ from NIKS in that they can be grown without a feeder layer for more than 15 passages.

Replicative Senescence

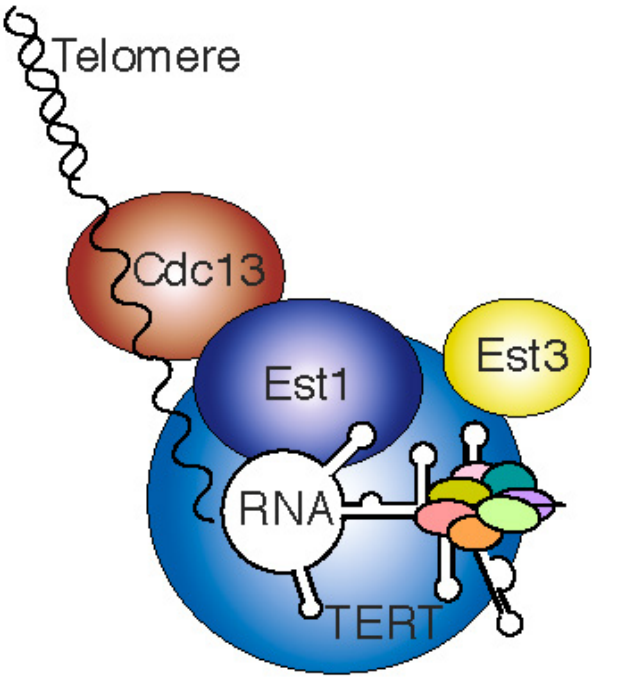
Telomeres

Telomeres are regions of repetitive DNA that cap the ends of chromosomes, preventing loss of vital genetic information when cells divide. Telomeres are presumably an adaptation evolved by eukaryotes who, unlike their prokaryotic ancestors, had to replicate linear instead of circular chromosomes.

There is a consistent shortening of telomeres during cellular aging. Human telomeres shorten by about 100 bp per cell division. When several kb of telomeric DNA are lost, this triggers the cell to undergo senescence. An interesting line of research lies in understanding how this triggering occurs, since it is presently unknown. It is not that senescent

human cells have lost all their telomeric DNA; they carry residual telomeres similar in length to those of many other eukaryotes.

A hypothesis for how telomeric loss triggers senescence deals with the formation of abnormal chromosome structures called dicentric and ring chromosomes.[33] These structures have been observed at senescence at a relatively high frequency (30-70%), when a critical telomere length has been reached. It has been proposed that the loss of telomeric DNA from any chromosome generates dicentric and ring chromosomes. These unstable structures break during the next mitotic event, signaling a DNA damage pathway that ends with G1 cell cycle arrest and senescence. The frequency of dicentric chromosomes dramatically rises near senescence and coincides with p53 protein activation, suggesting that the formation and breakage of dicentric chromosomes activates p53. As mentioned earlier, senescence is mediated by p53 activity.[34]



Speculative model of yeast telomerase

From Dr. Katherine Friedman's Lab, Vanderbilt University
http://www.molbio.vanderbilt.edu/mbdept/faculty/friedman.html

In animals, long-lived species often have shorter telomeres than short-lived species, indicating that telomere length probably doesn't determine lifespan. Mice, for example, have telomeres 3 times longer than ours, yet its cells do not live 3 times as long. However, older people do have shorter telomeres than younger people.

Perhaps the observation that longer-lived species often have shorter telomeres than shorter-lived species results from better antioxidant mechanisms in longer-lived species, allowing them to achieve a longer life. Therefore, the telomeres don't get shortened as much in longer-lived species as they would in shorter-lived species with poorer antioxidant mechanisms. That might be why shorter-lived species have longer telomeres, to buy more time before oxidative damage provokes senescence.

The size of the telomeric G-rich 3' overhang is longer in cell strains whose telomeres shorten more rapidly in culture. This implies that end-processing events might be manipulable to slow the rate of shortening in normal cells.

Telomerase

Composition of Telomerase

Telomerase is a ribonucleoprotein with reverse transcriptase activity. It works by adding TTAGGG repeats directly to the G-rich 3' overhang, thus extending the length of telomeres. The catalytic subunit of human telomerase is called hTERT, which has reverse transcriptase activity.[35] hTERT is located at chromosome 5p15.33.[36] The RNA component of telomerase is encoded by hTR, human telomerase RNA, and is where the sequence complementary to TTAGGG resides. It is located at chromosome 3q21-q28.[37] A mammalian protein that is associated with telomerase, TP1, can be found at chromosome 14q11.2.[38]

Telomerase and Senescence

Telomerase protects against replicative, but not other forms of cellular senescence (DNA damage, oncogenes, etc.). It preferentially elongates the shortest telomeres, and it is the shortest telomeres, not average telomere length, that limits

proliferation. This supports the interpretation that DNA damage signals rather than telomere positional effects induce the senescent growth arrest.

The telomeres of somatic cells are significantly shorter than in germline cells. This is because the enzyme telomerase, which acts to lengthen the telomeres, is active in germline and stem cells. Without this enzyme, these cells would also senesce.

Telomerase and Immortalization

Since most somatic human cells do not express the reverse transcriptase but contain all other enzymatic components for telomerase, forcing the expression of the missing hTERT can reconstitute enzyme activity.[39] This was done in an experiment where human cells were transfected with the hTERT gene. As a result, the newly reconstituted telomerase lengthened telomeres with additional TTAGGG repeats. Many transfected cell lines proceeded for 20 population doublings beyond their normal senescence point, grew vigorously, and showed a normal karyotype and youthful morphology. They continued to exhibit normal checkpoint controls, could be growth arrested by the overexpression of oncogenes like H-ras, and did not form tumors. This outcome occurred in retinal epithelial cells, foreskin fibroblasts, and vascular endothelial cells.[40]

In epidermal keratinocytes and mammary, adenoid, thyroid, and prostate epithelial cells, telomerase is insufficient for immortalization. Inactivation of the p16/pRB pathway by methylation of the p16 gene or expression of viral oncogenes like human papilloma virus protein E7 is also required. However, many of these cells are grown in a chemically-defined medium and not on feeder layers. If grown on feeder layers, they can be immortalized by telomerase without inactivating p16. Thus, the medium matters. If an immortal cell line can grow without a feeder layer, perhaps it has the p16/pRB pathway or something like it inactivated.

Telomerase and Cancer

Telomerase is turned off in most human tissues during development, except for germline and stem cells. One or more tumor suppressor genes may prevent activation of telomerase in normal human cells. To become immortal, cells must undergo a crucial change in telomere dynamics. They must either activate telomerase or an alternative mechanism that maintains telomeric DNA (ALT pathway). This is necessary for an immortal phenotype because without telomere maintenance, replicative senescence would eventually occur.

We have seen that the forced expression of telomerase can create immortal but otherwise normal, non-tumorigenic cells. To form a tumor, at least 3 additional functions are required: H-ras to activate autonomous cell division, SV40 large T antigen to block pRB and p53 checkpoint/apoptotic mechanisms, and SV40 small t antigen to inhibit phosphatase activity.

Tumors also have much higher telomerase activity than that occurring normally in stem cells. Telomerase is usually sequestered after cell division, but in cancer cells, telomerase is often present throughout the cell, all the time.

Oxidative Damage

Mitochondria and Oxidative Damage

Mitochondria are the sites of ATP production through the process of oxidative phosphorylation. Because of the oxidative, energy-yielding set of reactions required to make ATP, they are also an important source of reactive oxygen species and other free radicals. Since these reactive byproducts of cellular respiration are produced so close to the mitochondria, the mitochondria have a much greater likelihood of being damaged by these byproducts than other cellular components. Their DNA consists of circular strands of 16,569 nucleic acids that code for 13 proteins. Because many mitochondrial genes have been lost or relocated to the nucleus over the course of evolution, the mitochondria have poorer DNA repair capabilities than the nucleus. This compounds the problem of increased damage to this important organelle.

Cells have multiple protective mechanisms, and can prevent damage if the oxidative stress is not too great. However, any imbalance between pro-oxidant and anti-oxidant systems can lead to oxidative damage. Free radicals can damage the mitochondrial membranes, proteins, and DNA, leading to decreased energy-producing efficiency over time. In addition, damage to DNA repair and antioxidant genes hampers their ability to function. Oxidative damage to genes will go unrepaired and free radicals will go unquenched. These in turn will cause more damage to DNA repair and antioxidant genes. It is a vicious cycle, and one that contributes greatly to the aging process.

Oxidative damage is the main source of mutation and structural degradation in cells. Reactive oxygen species can diffuse rather far from their site of formation, whether they were made in the mitochondria or other sources such as oxidases in the cytoplasm. Without enhancing DNA repair and antioxidant machinery, even replicatively immortal cells will eventually succumb to oxidative damage. Simply turning on telomerase is not a cure-all for aging. Even with much improved DNA repair and antioxidant systems, some free radicals will undoubtedly escape to create non-recyclable byproducts that will accumulate within the cell, hampering its function. Thus, it is certainly possible today to extend life by manipulating genes, but without more advanced tools that are only in the earliest stages of development (e.g. nanotechnology), it will not be possible today to extend life indefinitely.

Mitochondrial Retrograde Response and Lifespan

Nuclear gene expression can be modulated by changes in the functional state of mitochondria. This modulation takes place through a communication pathway between the two organelles called the retrograde response. This retrograde signaling pathway functions as a homeostatic or stress response mechanism to adjust various biosynthetic and metabolic activities to changes in mitochondrial activity.[41]

Of special importance are changes in the expression of senescence or lifespan-extending genes via the retrograde response. The products of three yeast genes RTG1, RTG2, and RTG3 are expressed in yeast with defective mitochondria, which also happen to have increased longevity. These genes signal the nucleus that the mitochondria are less than fully functional in the retrograde response.[42] Adding antioxidants to the culture media do not increase their lifespan, which suggests that reduced oxidative damage is not the mechanism for life span extension in these cells. Instead, lifespan extension and delayed aging appears to be triggered by the products of the RTG genes, which trigger the retrograde response in the cell nucleus.

Free Radical Types

There are five main types of free radicals, all with varying degrees of toxicity. They are nitric oxide, peroxynitrite, hydrogen peroxide, superoxide anion, and the hydroxyl radical. Of the five, hydroxyl radical is the most reactive. All cells have some mechanisms of detoxifying these radicals and repairing the damage they cause. It is only when the production of radicals exceed the cell's capacity for detoxification that oxidative damage occurs, which unfortunately happens quite frequently.

NO radical (nitric oxide) either reacts quickly with oxygen, or can react with superoxide anion to form the more reactive **peroxynitrite**, which can decompose to form a hydroxyl radical. Peroxynitrite attacks protein cysteines, methionines, and tyrosines by adding an NO_2 to the ring of these amino acids. These protein modifications may explain the observed biological effects of this radical. Nitric oxide is involved in smooth muscle relaxation, neurotransmission, and immune regulation. However, elevated levels of nitric oxide can be toxic to DNA[43] and may even cause cancer.[44]

H_2O_2 (hydrogen peroxide) is a non-radical that can diffuse over considerable distances, and may pass membranes readily in the process.

O_2 radical (superoxide anion) is continuously generated by several cellular processes, including the microsomal and mitochondrial electron transport systems. Oxidation processes involving cytochrome p450 and NADPH-cytochrome c reductase on the endoplasmic reticulum can generate superoxide anion and H_2O_2. Xanthine dehydrogenase/oxidase and other cellular oxidases are also important sources. Superoxide anion is not a particularly reactive molecule, and can diffuse considerable distances from its varied sites of production. The superoxide dismutase enzyme (SOD) universally present in both cytoplasm and mitochondria ensures that much of this anion is rapidly converted to hydrogen peroxide and O_2.

In the presence of a transition cation like Fe or Cu, superoxide anion can give rise to the highly reactive **hydroxyl radical** species (HO.). HO can react with biomolecules and produce products that cannot be regenerated by cell metabolism. The hydroxyl radical reacts very close to its site of production, making its damage very site-specific.

When HO reacts with nucleic acids, it modifies the bases. Examples of HO base attack products are 8-hydroxyguanosine, thymidine glycol, and uric acid.

When HO reacts with proteins, they can be fragmented. This fragmentation is associated with reactions at specific amino acids such as proline and histidine. Proteins can also contain metal binding sites that are especially susceptible to oxidative events through interaction with the metals. These reactions usually produce irreversible modifications in amino acids involved in metal ion binding (i.e. histidine), and may create signal sequences recognized by specific cellular proteases that degrade such proteins.

Cysteines and methionines on intracellular proteins can be reversibly modified by radicals. This serves a protective role since the radical is detoxified without causing harm. Oxidation of proline, however, is irreversible. The result of such oxidation is a break in the polypeptide chain and the introduction of new carboxyl groups.

Lipid Peroxidation

Lipid peroxidation is a radical-initiated chain reaction that self-propagates in cell membranes, occurring on unsaturated fatty acid chains that reside within the lipid bilayer. It is mainly what causes rancidity in foods and decaying animals. Lipid peroxidation of polyunsaturated lipids occurs easily by reacting with hydroxyl radicals, which yield lipid radicals. When two lipid radicals meet, they can detoxify each other, but only by forming a covalent bond. As a result, lipid radicals can seriously impair membrane function by making the membrane rigid and reactive to other biomolecules.

The most commonly measured product of lipid peroxidation is malondialdehyde. The unsaturated aldehydes produced by these reactions are implicated in modifying cellular proteins and other materials.[45] A peroxidized lipid can produce peroxy radicals and singlet oxygen.

Vitamin E, a lipophilic molecule, is a particularly effective antioxidant in biological membranes and lipid particles found in blood plasma. However, it is not 100% effective, as is any antioxidant, from quenching all free radicals that might produce damage to cellular components.

Oxidative Detoxification and DNA Repair

These are the only two defenses cells have at their disposal for combating oxidative damage. Antioxidants try to prevent the damage before it occurs, while DNA repair mechanisms try to fix the damage. Neither are 100% effective, as endogenous and dietary antioxidants are not available at high enough concentrations to neutralize all free radicals. The ones that escape will eventually cause some kind of irreversible damage to biomolecules. Improving these systems and perhaps designing better antioxidants, such as one made at Stanford University that can localize itself to mitochondria 80 times more effectively than vitamin E, will extend the time before oxidative damage triggers cellular senescence or cancer.

Superoxide dismutase (SOD) neutralizes superoxide anion radicals by converting them to H_2O_2. Catalase and glutathione peroxidase then neutralize the H_2O_2. In Down's syndrome, there are 3 copies of the SOD gene because it resides on chromosome 21. With the overproduction of SOD, catalase and glutathione peroxidase can't keep up with the accelerated production of H_2O_2. The hydrogen peroxide thus accumulates in the cells, causing increased oxidative stress, free-radical proliferation, and accelerated aging. An excess of SOD leads to the premature oxidation and death of brain cells. The degeneration of neurons in Down's cells has been shown to be preventable by applying antioxidants.

SOD is present as a Cu-Zn enzyme in the cell cytoplasm, and as the Mn enzyme called MnSOD in the mitochondria. It needs a Cu chaperone to cross the membrane. The absence of SOD is lethal. The amount of SOD in the cell is controlled by specific redox-sensitive genes. There is also an extracellular form of SOD in plasma, lymph, and synovial fluid that differs from the intracellular form. The extracellular enzyme may function at cell surfaces.

Overexpression of MnSOD (also called SOD2), along with other forms of SOD has been shown to increase the lifespan of adult *Drosophila melanogaster*.[46] However, MnSOD overexpression in yeast increases the chronological lifespan, but dramatically reduces replicative lifespan. This could be due to the absence of adequate mechanisms in yeast mitochondria to deal with the increased H_2O_2 that more MnSOD would produce.

Catalase is a heme protein that detoxifies H_2O_2. It is usually found in peroxisomes except in cells like erythrocytes that don't have them. In that case, catalase is a cytoplasmic enzyme. Catalase along with glutathione peroxidase, a cytoplasmic and mitochondrial enzyme, are important in detoxifying H_2O_2.

Glutathione is a peptide made from the amino acids cysteine, glycine, and glutamic acid that detoxifies H_2O_2. It is found at high intracellular concentrations and has high water solubility, and is the major antioxidant in much of the cytoplasm.

Glycosylases, exonucleases, and endonucleases are among the group of enzymes that repair DNA.[47]

How Oxidative Damage Affects Telomeres

Hyperoxia can induce single-stranded breaks in the G-rich strand of telomeres, which are repaired much less efficiently than those elsewhere in the genome. As a result, the telomeres shorten, and G-rich single-stranded DNA fragments begin to accumulate. This accumulation of ssDNA due to telomere shortening triggers a p53-dependent cell cycle arrest.

Reactive oxygen species have been shown to increase in concentration during the last 15 doublings of IMR90 lung fibroblasts in vitro. This may explain observations that telomere shortening appears to be caused by oxidative damage during the last 15 doublings in similar cells. The rate of shortening can be slowed by introducing antioxidants into the medium.

Oxidative damage to telomeres produces breaks of random sizes and does not affect the size of the G-rich 3' overhang. The rate of telomere loss in different cultured cell types is proportional to the size of this overhang, strongly suggesting that events at the end of the chromosome (the end-replication problem and processing mechanisms) are the primary determinant of the rate of telomere shortening.

Cell Culture Medium and Damage

Cell culture media can often induce DNA damage. Proliferation has been shown to stop after only 3-4 doublings in mouse cells containing defects in a large variety of DNA repair functions, but since the animals are viable, they must be able to divide much more than 4 times in vivo. Interestingly, mouse cells have been reported to be immortal and remain diploid if cultured in a special medium.[48]

Maybe mice don't have telomere-based replicative senescence, but culture-induced growth arrest instead. Still, TBRS could have evolved in larger, longer-lived organisms as an additional restraint against tumor formation.

Structural Degradation and Genetic Instability

The first obvious cause of structural degradation is oxidative damage to biomolecules. We have seen that reactive oxygen species can attack lipids, proteins, carbohydrates, and nucleic acids, causing the destabilization and dysfunction of cellular architecture. Environmental causes of structural degradation such as high temperature, changes in the extracellular chemistry, and physical stress such as excessive movement or even pressure in the form of intense noise[49] can act by producing oxidative damage. One other important cause of structural degradation is a change in the expression of genes that contribute to structural integrity.

Structural degradation has a direct link to genetic instability, and thus to senescence. Researchers at Duke University have found that genetic instability can be caused by stresses that do and do not directly damage DNA.[50] For example, if the cytoskeleton supporting the nuclear membrane or nucleolus is compromised either by environmental stress, oxidative damage, or genetic causes, this will signal cell cycle arrest via CDK inhibitors, which will benefit the cell by preventing an otherwise problematic cell division. This is because if spindle fibers cannot attach to the cytoskeleton properly, cell division will likely go awry. Especially compelling evidence for a structural contribution to aging can be found in Werner's syndrome and progeria. Here, the loss of a gene that contributes to nucleolar or nuclear membrane structural integrity is responsible for rapid aging. With poor structural integrity in the nucleus, cells in these patients are triggered to senesce much earlier than usual through the usual senescence pathways.

It is quite interesting that senescing plant leaves exhibit many of the same structural changes as senescing animal cells. As a plant or its leaves get older, the leaves show increasing photosynthetic rates (growth) until the time of flowering (reproduction), after which the rates fall off rapidly, regardless of the age of the individual leaf. The aging of leaves is associated with an extended decline in the protein and chlorophyll content, an increase in leaf permeability, and an increase in the apparent free space of leaf tissues, which implies an increase in the leakiness of membranes in the leaf. There is also structural deterioration of chloroplasts, and much later, the nuclear membrane.[51]

Several sources cite genomic instability as a cause of aging instead of gene dysregulation.[52] There is also much evidence that damaged proteins play a role in aging, and that overexpression of chaperones can extend lifespan.[53]

Werner's Syndrome: Degradation of the Nucleolus

The nucleolus contains highly repeated copies of rDNA. The repeated nature of this rDNA may make it less stable than the rest of the genome, and more vulnerable to the fragmentation seen in older cells.[54] During cell reproduction the nucleolus disappears, but not all of its fragments may disappear, particularly the extrachromosomal rDNA circles (ERCs) formed by homologous recombination. It is known that ERCs get replicated until they reaches toxic levels in old cells, but the mechanism of toxicity is not known.[55] It is hypothesized that these fragments remain and hamper the formation of spindle fibers during mitosis, which may contribute to the greater risk of chromosomal abnormalities as cells age and accumulate ERCs. Some proteins that localize themselves in the nucleolus may counteract homologous recombination, thus preventing the formation of mitosis-hampering ERCs. WRN may be one such protein.

WRN is the protein defective in Werner's syndrome, a premature aging disorder that causes cataracts, osteoporosis, diabetes, and cancer at an early age. The WRN gene product is located in the nucleolus, and has 3'-5' helicase and 3'-5' exonuclease activities.[56] [57] It has been shown to be recruited by TRF2, a telomeric repeat binding factor, to facilitate the breakdown of telomeric fragments.[58] TRF2 by itself stabilizes telomere ends by promoting the formation of stable T loops, prevents end-joining, and prevents the activation of cell checkpoint pathways that unprotected telomeric ends

would cause. Without WRN, unusual structures on telomeres cannot be degraded properly, which can cause senescence. It is notable that the addition of telomerase can restore the lost telomere maintenance function of WRN and immortalize Werner's cells.

However, WRN may have other functions in the nucleus, as evidenced by its localization within the nucleolus. The mouse homolog of WRN, mWRN, is present diffusely throughout the nucleus in mice instead of localized within the nucleolus. Mice live shorter lifespans than humans (2 years vs. an average of 80 years), and appear to die of cancer rather than old age.[59] This would seem to suggest that WRN interacts with nucleolar components in a way that extends lifespan.

Certain genes in yeast determine lifespan, promoting cell longevity by moving from the telomeres to the nucleolus. The yeast longevity gene SGS1 corresponds structurally to human WRN. Experimental mutation of SGS1 produced symptoms of aging in yeast, the foremost of which was fragmentation and enlargement of the nucleolus. A mutation in SGS1 shortens yeast lifespan by ~60%, a striking parallel to Werner's. These results suggest that nucleolar fragmentation may be a cause of aging. They do not specifically suggest that ERC formation might be the cause of this aging, so this would be another avenue of research. It would seem, however, that nucleolar fragmentation and enlargement would be consistent with the accumulation of extrachromosomal rDNA circles. Also, the preponderance of these and other non-recyclable cellular byproducts may contribute to the larger size of older, senescent cells compared with younger cells.

It is interesting that SGS1 and WRN both move from the telomeres to the nucleolus. This protein that normally resides on the telomeres must somehow be triggered to move to the nucleolus instead. If the telomeres are deacetylated, as they would be if the histone deacetylase and known lifespan-extending enzyme SIR2 were active, the chromatin would condense near the telomeres. This chromatin condensation may trigger the release of SGS1/WRN, perhaps because the substrates they normally attach to are no longer accessible or have already been released during chromatin condensation. Without binding spots available at the telomeres, this protein would have a greater chance of being found in the nucleolus where it can suppress nucleolar fragmentation.

Another line of research has shown that the concentration of WRN in the nucleolus remains constant in a variety of normal and cancerous human cells, indicating that the presence of other diseases does not disrupt WRN from its job.[60]

Sir2 is also known to extend yeast lifespan by stabilizing repetitive DNA.[61] Resveratrol was able to reduce the frequency of rDNA recombination by about 60% in a SIR2-dependent manner.

Progeria: Degradation of the Nuclear Membrane

Progeria is a premature aging disorder caused by a mutation in the lamin A gene (LMNA), which codes for a key component of the nuclear membrane. LMNA is located at 1q21.2. Eighteen of 20 classic cases of progeria showed a C-to-T substitution at codon 608 within exon 11 of LMNA, resulting in a silent gly-to-gly change (G608G). One additional case had a G-to-A substitution just two bases upstream in the same codon. In all cases, the parents were normal, so the misspelling was a new mutation in the child. Both mutations activated a cryptic splice site within exon 11, producing a protein product with 50 AA deleted from its C terminus. This defective lamin A still retains the CAAX box, but lacks the site for endoproteolytic cleavage.

Lamin A is a key component of the nuclear membrane. It is important to note that in these cases of progeria, **many cells showed visible abnormalities of the nuclear membrane.** Most cells had strikingly altered nuclear sizes and shapes, with envelope interruptions accompanied by chromatin extrusion. Lamin A levels were at 25% of normal in this case.[62] The resulting instability of the nuclear membrane is problematic for tissues subjected to intense physical stress, such as cardiovascular and musculoskeletal tissues.

Different mutations in other regions of LMNA lead to Emery-Dreifuss muscular dystrophy type 2, limb girdle muscular dystrophy type 1B, Charcot-Marie-Tooth disorder type 2B1, Dunnigan type of familial partial lipodystrophy, mandibuloacral dysplasia, and familial form of dilated cardiomyopathy.

Degradation of the Extracellular Matrix

Matrix metalloproteinases, collagenases, and interleukins are among the proteins secreted in increased amounts by senescent dermal fibroblasts. These extracellular proteins degrade the extracellular matrix architecture, causing degradation of connective and other tissues as senescent cell populations increase with age. Both the collagenous and elastic components of dermal connective tissue display degeneration consistent with the overexpression of proteolytic activity.

Alpha1(I) and alpha 2(I) collagen mRNAs are significantly lower in senescent fibroblasts and lowered by the same extent, which implies coordinate regulation of the two genes. Type I collagen is the most abundant in skin. The

secretion of type I and III collagens decrease linearly with age, though there is always the same ratio of the two collagen types. There are no age-related variations in cell-associated collagen, however.

The altered extracellular matrix composition can affect pre-senescent cells via signal transduction at the cell surface. It is possible that these signal transduction pathways could alter gene expression in pre-senescent cells, perhaps accelerating their time to senescence.

Caloric Restriction Alters Senescence Gene Expression

CRAN stands for caloric restriction with adequate nutrition. It is the only reproducible method of extending lifespan in a wide variety of short-lived species, and lately has also been shown to produce similar metabolic changes in longer-lived primates.[63] Lowering calorie intake essentially lowers the amount of glucose available to mitochondria for cellular respiration, and thus it must be here where CRAN's effects originate.

Cellular respiration consists of glycolysis in the cytoplasm, and the Krebs cycle and electron transport chain (ETC) on the inner mitochondrial membrane. Glycolysis involves the breakdown of glucose to 2 molecules of pyruvate, and the reduction of the electron carrier NAD+ to NADH. The pyruvate is transported across the mitochondrial membrane to where the Krebs cycle occurs. Here, more NADH are made than in glycolysis. Finally, the ETC oxidizes the NADH back to NAD+, using their electrons to eject protons into the matrix, creating an electrochemical gradient that forces them back through the inner mitochondrial membrane. The protons can only re-enter through ATP synthase transmembrane proteins, thus driving the synthesis of ATP which is used by the cell for energy.

The feature of cellular respiration important to CRAN is NADH. The NADH molecules go through the electron transport chain to ultimately make ATP. NADH accumulates when the cell is under anaerobic conditions and can't use the ETC to re-oxidize NADH back to NAD+. When calories are restricted, fewer NADH enter the ETC, which limits the oxidation of NADH back to NAD+.[64] It turns out that CRAN extends lifespan by lowering the level of NADH in the cell, which causes the activation of a special gene.

This special gene is SIR2, and without it, the lifespan extension of CRAN cannot occur.[65] SIR2 which works by silencing genes that promote senescence.[66] The next section discusses SIR2 in more detail. It was once thought that the increase in NAD+ concentration activates Sir2p, the protein product of SIR2.[67] However, Lin *et al.* (2004) recently showed that although the NAD+/NADH ratio did contribute to the activation of Sir2p, it was **actually a decrease in NADH, not an increase in NAD+, that caused the activation**.[68] During CRAN, NAD+ levels are high in the cytoplasm and nucleus, so NADH levels must be low, thereby activating more SIR2.[69]

Not only does CRAN extend lifespan by suppressing senescence, but it also lessens the amount of oxidative damage in cells. Less glucose to burn means fewer free radicals are produced.

Other Features of Caloric Restriction[70]

- CRAN increases apoptosis, which generally decreases with age in mitotic tissues. The basal rate of apoptosis in resting tissues eliminates dysfunctional cells.
- There is a reduction in the disulfide bonds in non-histone chromatin proteins, which promotes more transcription compared to normal aging animals.
- There are higher levels of DNA repair, and much higher levels of gene expression. Protein synthesis declines more slowly with age than it normally does, and in most organs except skeletal muscles and lungs, protein synthesis actually increases.
- Lower body temperature is not the cause of CRAN's effects, nor is the immune system. There are fewer leukocytes, lymphocytes, and antibodies, but more cell-mediated T-cell killing of tumor cells.
- The thyroid hormones T4 and T3, which is more potent, increase O2 consumption in almost all tissues, except brain, testes, uterus, lymph nodes, spleen, and anterior pituitary. In CRAN, there is a drop in serum T3 to less than half that of control animals, but total body T3 (including that bound to proteins) is the same in both groups.
- There is a 15% reduction in blood glucose in rats. This could mean less protein cross-linking by glucose, which is one possible contributor to aging. There is less collagen aging by cross-linking.
- SOD activity does not increase w/ CRAN. In fact, brain homogenates show less SOD and lysosomal enzymes. The antioxidant glutathione increases somewhat. Catalase increases very dramatically; it typically falls w/ age.
- The likely causes of CRAN are selective upregulation in repair and protective processes, increased metabolic efficiency, decreased production of damaging agents, and signals to the neuroendocrine network, particularly the hypothalamus. The causative events of CRAN likely take place in proliferative systems (dividing cells).

Also, reduced glycation (protein cross-linking by sugars) and reduced free radical generation could be other contributors to CRAN's effects.

CRAN Decreases Senescence Gene Transcription via SIR2

What is SIR2?

SIR2 (silent information regulator) is a histone deacetylase necessary for transcriptional silencing at the telomeres. It functions in a complex with SIR3 and SIR4. These three genes along with UTH4 determine lifespan in yeast. If these genes are deleted, yeast lifespan is significantly shortened. If these genes are overexpressed, the lifespan extends way beyond wild-type. The gene products encoded by SIR2, 3, and 4 move from the telomeres to the nucleolus; it is this action that promotes longevity.

Sir2p (the protein encoded by SIR2), along with Sir1, 3, 4, and Rap, deacetylates the N-terminal tails of histones H3 and H4 at telomeres, one of the 3 deacetylation sites in yeast.[71] They extend lifespan by silencing genes.[72] When the acetyl groups are moved from the histone tails, they become positively charged and interact more strongly with negatively charged DNA molecules. The DNA/histone complexes become more tightly packed, which cause the chromatin in that region to become more tightly packed, which causes transcriptional repression.[73] Since genes near the ends of telomeres are likely inactivated due to the tight packing of telomeric chromatin, this supports the idea that genes at the ends of chromosomes are responsible for promoting senescence.

SIR2 also silences and represses mitotic recombination of rDNA by establishing a more closed chromatin structure there.[74] By suppressing rDNA recombination, SIR2 stifles the production of extrachromosomal rDNA loops that get replicated to toxic levels, which would otherwise trigger cellular senescence.[75]

A SIR2 mutation can shorten lifespan by 50% because the mutants can't suppress the generation of ERCs.[76] ERC accumulation in yeast is a cause of aging.[77] Sir2p localizes to the rDNA and represses homologous recombination, which is necessary for ERC formation. Since the SIR2 mutants can't suppress ERC generation, they age faster and have a shorter lifespan.[78]

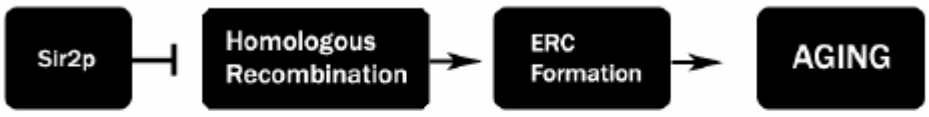


Figure 1. Sir2p linked to yeast aging. Active Sir2p represses homologous recombination. Normally, homologous recombination is required for ERC formation and aging. However, this function is knocked out in yeast that express Sir2p. Therefore, ERC formation does not occur and cannot stimulate this particular aging pathway.

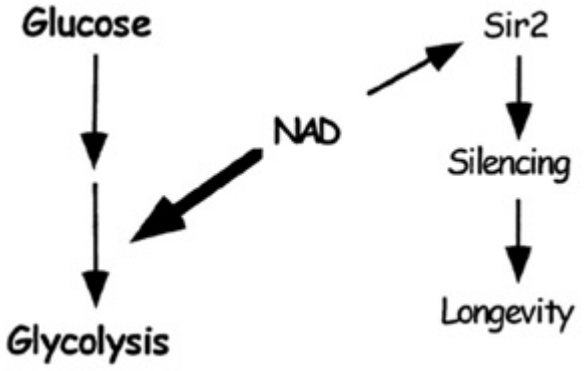
From Caroline Lindsay SIR2 Protein: An Important Link Between Aging and Metabolism http://sq.ucsd.edu/sq1_caroline_lindsay.pdf

SIR2 also has a weak ADP-ribosyltransferase activity.[79] ADP-ribosylation is associated with the repair of damaged DNA. DNA damage causes the rapid induction of polyadenosine diphosphate ribose synthesis (ADP-ribosylation) in nuclei, which results in the extensive depletion of cellular NADH pools. SIR2, which requires low NADH, is likely activated. This would be appropriate since Sir2- is also known to function in dsDNA break repair.[80]

The Link Between SIR2 and Senescence

Histone deacetylase inhibitors such as SIR2, which decondense chromatin and activate the transcription of some genes, have already been shown to induce a senescence-like state in human fibroblasts.[81] [82] This suggests that the conversion of some heterochromatin (condensed, inactive DNA) to euchromatin (actively transcribed DNA) may be a feature of replicative senescence.

The HDAC inhibitor sodium butyrate rapidly induces a senescent-like phenotype in human fibroblasts[83] and reduced levels of Sir2, a protein with HDAC activity, causes accelerated aging in yeast cells.[84]



In the presence of HDAC inhibitors, fibroblasts senesce after several population doublings.[85] This state resembles replicative senescence in terms of morphology, saturation density, and cell cycle distribution, including the accumulation of hypophosphorylated RB and induction of CDKI $p21^{Waf-1}$ protein.[86] In yeast cells, overexpression of Sir2 extends life span in a manner dependent on NAD-dependent HDAC activity.[87] It is presently unknown whether mouse or human Sir2 homologues have similar activity.

SIR2 and the Insulin-Like Signaling Pathway

Increasing the expression of the sir-2.1 gene extended the *C. elegans* lifespan, as overexpression of SIR2 did in yeast.[88] In *C. elegans*, lifespan is partially controlled by the insulin-like signaling pathway.[89] Insulin signals the cell to produce more glucose, stimulating more cellular respiration and leading to more NADH production. Insulin is important in regulating the NAD+/NADH ratio needed for both Sir2p and sir-2.1 activity. The sir-2.1-dependent lifespan extension is also dependent on daf-16, one of the downstream genes in the insulin-like signaling pathway.[90]

Conclusion

There is much more knowledge about the aging process than has been included here, since this paper was meant only to be an overview of what we currently know. However, it is a good start for those who might not have heard of much credible research on aging, as well as for biomedical researchers, who may want to contribute their research and experience toward the goal of extending human lifespan.

Reaching this goal will require collaboration across many different fields of biology and medicine, including but not limited to genetics and genomics, molecular and cell biology, biophysics, structural biology, cancer biology, and biochemistry. It will also require the adaptation of current technologies toward new uses. A brief proposal of how available technology might be adapted toward this goal is discussed below.

Extending Lifespan with Gene Therapy

The first technology we should consider in reaching our goal of extending human lifespan is gene therapy. In particular, adenoviral gene therapy is a reliable technology that can be put to work today. Past methods of gene therapy have largely been ineffective and in some cases have resulted in severe side effects. However, modern adenoviral approaches are being worked on at many universities, and have been shown to be safe and reliable in human cells.

Adenoviral vectors can insert genes into a particular spot within the genome without triggering the immune system or causing other harm. With our knowledge of DNA structure, we will likely want to have new genes inserted into stretches of DNA that are usually in the form of actively transcribed euchromatin rather than inactive, condensed heterochromatin. These new genes will either be repressors of senescence, or promoters of long lifespan by decreasing oxidative damage and/or stabilizing nuclear structure. We will likely want to insert genes that do both, to derive maximum benefit.

Success in gene therapy for aging must be shown in lower eukaryotes before any steps can be made to treat humans. Thus, it is important to start early with attempts in yeast, C. elegans, fruit flies, and mice. A mouse genetically altered to have longer lifespan would especially attract interest from more researchers since it is a species not too distantly related to our own. Such a mouse would certainly stimulate a push for trials in humans.

The Technological Challenge of Treating Older Humans

It is the older population who could stand to benefit the most from such lifespan-extending gene therapy. However, an obstacle unique to the elderly is the structural and genetic deterioration already present due to the high concentration of senescent cells. Gene therapy against aging would be most effective if done to prevent senescence in the first place; thus younger people would stand to benefit more from this treatment. It will not be possible to stop the senescence process in the elderly, whose cells have mostly already undergone senescence. To treat the elderly, who have accumulated much damage to their cells over the years, senescent cell populations would need to be culled and replaced by healthier, pre-senescent cells that have been stimulated to divide.

One piece of the technology to do this can be found in labs where new tissues have been stimulated to grow from adult stem cells. These techniques can be used to grow new tissues if a healthy cell from the body can be found. Another piece can be derived from cancer research, where researchers have targeted genes unique to cancer (e.g. telomerase)[91]. Senescent cells display an altered expression of proteins on their cell surface, making them targetable by both viral vectors and the body's own immune system. Viral vectors could be used to insert genes that would lead to apoptosis of senescent cells,

while anti-senescent cell antibodies could be used to dispatch these cells. The death and removal of senescent cells must take place slowly, and be balanced with the regrowing of new tissue to avoid any adverse effects caused by rapid cell death and renewal.

A Call to Action

While seemingly immortal cells continue to flourish, dividing fruitfully in their scattered laboratory Petri dishes, we capable human beings are all destined to die without exceeding today's maximum lifespan. This is the case only because little initiative has been taken to try and extend human life.

The technology currently exists to enable people to enjoy extra healthful decades of life. We could have more time to experience life, learn from it, teach others what we have learned, and prepare for its conclusion. If given extra years to live, researchers may find ways of extending lives even further, just as Einstein could have figured out a quantum theory of gravity if he did not run out of time. Humanity will very likely continue to be resourceful in finding technological solutions to problems, but we may not live long enough to see these solutions.

It has always been difficult to invest time and resources into something whose results may never be seen, although people have been doing this throughout history. We plant trees in our old age, knowing we will never sit beneath their bountiful shade. We teach our children to be good citizens so they can take over things after we are gone. We set aside forests and preserves so that our posterity will have some knowledge of how beautiful the world can be without human interference. In the same spirit, we can choose to pour funding into lifespan-extending research. Those who have amassed great wealth can choose to spend more of it on research that could lengthen and enrich the quality of their aging lives.

In the end, perhaps our own years may not be lengthened, but those of our children might. It says much about the character of a person willing to do this, that he or she really cares about our future.

Academic Labs Conducting Research on Aging

The following are labs where research on the fundamentals of aging is being done. It was a list I compiled in search of good graduate labs and programs in this field. If you are interested in studying solutions to aging in graduate school, inquire with one or more of the professors below. Unfortunately it is only a list of American labs, though there is booming international research in the UK and other countries. I will try to compile an international list of labs soon, to support broader collaboration between countries on the issue of aging. Aging, of course, is a disease with no national boundaries. We could all benefit by sharing what we know about it, and possible techniques of treating it.

Duke University

Laura Rusche
lrusche@biochem.duke.edu
919-684-0354
http://ives.biochem.duke.edu/Rusche/rusche.html#Research%20Interest
formation and function of repressive chromatin in S. cerevisiae; Sir proteins

Christopher Counter
count004@mc.duke.edu
919-684-9890
http://pharmacology.mc.duke.edu/viewresearch.asp?id=46
telomerase; investigate how cancer cells acquire the ability to divide indefinitely, or become immortal

Tso-Pang Yao
yao00001@mc.duke.edu
919-613-8654
http://pharmacology.mc.duke.edu/viewresearch.asp?id=63
uncover novel functions for reversible protein acetylation in signal transduction (p53, HDACs studied)

Sally Kornbluth
kornb001@mc.duke.edu
919-613-8624
http://pharmacology.mc.duke.edu/viewresearch.asp?id=48
regulation of complex cellular processes, including entry into mitosis and apoptosis

Daniel Lew
daniel.lew@duke.edu
919-613-8627
http://www.duke.edu/web/lewlab/publications.html
http://pharmacology.mc.duke.edu/viewresearch.asp?id=50
cell cycle control, in particular how it interacts with control of cell polarity; CDKs and cytoskeleton

Dennis Thiele
dennis.thiele@duke.edu
684-5776
http://pharmacology.mc.duke.edu/viewresearch.asp?id=78
heat shock transcription factors, genome-wide stress responses, how Cu is acquired/distributed (important co-factor) to maintain homeostasis

Andrea Amalfitano
amalf001@mc.duke.edu
(919) 684-2036 ext. 0
https://faculty.duke.edu/faculty/info?pid=3570
http://www.duke.edu/~mdfeezor/dukemedicalgenetics/mgresearch.htm#amalfitano
gene therapy with adenovirus vectors

Tannishtha Reya
t.reya@duke.edu
919-613-8756
http://pharmacology.mc.duke.edu/viewresearch.asp?id=74
elucidate the signaling pathways (Wnt) that regulate (hematopoietic) stem cell fate

Antonius VanDongen
vando005@mc.duke.edu
919-681-4862
http://pharmacology.mc.duke.edu/viewresearch.asp?id=60
understand how ion channels work at the molecular level; how cells control functional expression of ion channels

Joseph Nevins
j.nevins@duke.edu
(919) 684-2746
http://mgm.duke.edu/faculty/nevins/index.htm
E2F transcription factor, Myc oncoproteins, Myc and Ras signaling pathways together with the Rb/E2F pathway in control of cell proliferation and fate

Steven Haase
http://fds.duke.edu/db/aas/Biology/faculty/shaase
Control of cell cycle, DNA replication, and centrosome duplication in budding yeast

University of Washington at Seattle

Junko Oshima
picard@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/oshima.html
Elucidation of the pathogenesis of human progerias (diseases of premature aging), Werner's and Hutchinson Gilford Syndromes.

Peter Rabinovitch
petersr@u.washington.edu
http://depts.washington.edu/mcb/facultyinfo.php?id=127
DNA damage and genomic instability in aging (Werner's) and cancer

Brian Kennedy
bkenn@u.washington.edu
http://depts.washington.edu/mcb/facultyinfo.php?id=218
Nuclear organization, lamins, premature aging

Norman Wolf
normwolf@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/wolf.html
Study of aging as it affects the lens epithelium and bone (osteoblasts); the mechanism(s) and prevention of age related cellular changes; CRAN

Raymond Monnat
monnat@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/monnat.html
Mutagenesis, oxygen free radicals, aging (Werner's) and cancer

Karen Swisshelm
kswiss@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/swisshelm.html
Study of senes-associated genes (including retinoic acid receptor beta) that contribute to the prevention/treatment of breast cancer; Werner Syndrome

Terrance Kavanagh
tjkav@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/kavanagh.html
Oxygen free radicals and glutathione metabolism in environmental disease and aging

Stephen Plymate

splymate@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/plymate.html
Insulin-like growth factor (IGF) receptor, senescence-associated genes, and androgen in prostate cancer

May Reed
mjr@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/reed.html
Angiogenesis in aging; extracellular matrix

Dave Eaton
deaton@u.washington.edu
http://www.pathology.washington.edu/research/bioage/faculty/eaton.html
biochemical and molecular basis for species and interindividual differences in response to chemical carcinogens

Baylor College of Medicine

Scott Pletcher
713-798-5524
pletcher@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_pletcher.htm
http://www.imgen.bcm.tmc.edu/molgen/facultyaz/pletcher.html
experimental, theoretical, and computational approaches to study genetic mechanisms underlying the biology of aging; CRAN and environmental approaches

Vicki Lundblad
(713) 798-3454
lundblad@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_lundblad.html
http://www.imgen.bcm.tmc.edu/molgen/facultyaz/lundblad.html
Telomeres in yeast and humans

Si Yi Chen
(713) 798-1236
sychen@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_chen.htm
Gene therapy for cancer and AIDS research

Lawrence Donehower
(713) 798-3594
larryd@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_donehower.html
role of tumor suppressor genes (p53) in cancer and aging

Gretchen Darlington
(713) 798-1565
gretchen@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_darlington.html
definition of the molecular basis for longevity in three different long-lived mouse strains

Margaret Goodell
(713) 798-1265
goodell@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_goodell.html
Hematopoietic stem cells

Anil Jaiswal
(713) 798-7691
ajaiswal@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_jaiswal.html
determination of molecular mechanisms of coordinated activation of defensive genes in response to oxidative stress

Estela Medrano
(713) 791-1414
medrano@bcm.tmc.edu
http://www.bcm.edu/cmb/applicants/facres/faculty_res_medrano.html
regulation of aging and cancer of human melanocytes

University of Texas Health Science Center at San Antonio

Olivia Pereira-Smith
(210) 562-5069
SMITHO@UTHSCSA.EDU
http://www.uthscsa.edu/csb/faculty/pereira.html
replicative senescence, immortalization, complementation groups, chromatin remodeling, transcriptional regulation

Pamela L. Larsen
(210) 567-0608
larsenp@uthscsa.edu
http://www.uthscsa.edu/csb/faculty/larsen.html
Molecular genetics, aging, development, expression analysis, functional genomics, genetic and environmental interactions and C. elegans physiology.

Robert A. Marciniak
(210) 567-4777
marciniak@uthscsa.edu
http://www.uthscsa.edu/csb/faculty/camarciniak.html
telomerase, aging, Werner syndrome, cell regulation

James R. Smith
(210) 567-5067
smithjr@uthscsa.edu
http://www.uthscsa.edu/csb/faculty/casmith.html
aging, senescence, gene expression, cell growth, apoptosis

Jan Vijg
vijg@uthscsa.edu
http://physiology.uthscsa.edu/faculty/vijg.html
http://fgoa.stcbmlab.uthscsa.edu/
Func gen of aging, oxidative stress, metabolism (CRAN)

Arlan Richardson
(210) 567-0561
RICHARDSONA@UTHSCSA.EDU
http://www.uthscsa.edu/csb/faculty/richardson.html
Aging, transgenic mice, knockout mice, oxidative stress, DNA repair

Brian Herman, Chair
(210) 567-3800
HERMANB@UTHSCSA.EDU
http://www.uthscsa.edu/csb/faculty/herman.html
Apoptosis, mitochondria, cancer, aging, optical imaging

University of North Carolina at Chapel Hill

Shawn Ahmed
shawn@med.unc.edu
http://www.med.unc.edu/genetics/faculty_ahmed.htm
http://www.bio.unc.edu/ahmed/lab/
Telomere Replication and Germline Immortality in C. elegans.

Thomas Petes
tompetes@email.unc.edu
http://www.med.unc.edu/genetics/faculty_petes.htm
Yeast studies of regulation of genome stability, telomere length control, and meiotic recombination

Norman Sharpless
NES@med.unc.edu
http://www.med.unc.edu/genetics/faculty_sharpless.htm
Tumor Suppressor Genes, Genetics of Cancer and Aging

Virginia Commonwealth University

Lynne W. Elmore
(804) 628-0256
lelmore@vcu.edu
http://www.pathology.vcu.edu/research/holt_el/elmore.html
Molecular Mechanisms of Aging & Cancer

Shawn Edan Holt
(804) 827-0458
seholt@vcu.edu

http://www.pathology.vcu.edu/research/holt_el/holt.htmlhttp://www.vipbg.vcu.edu/hg/dr/SHOLT.shtml
Molecular Mechanisms of Aging and Cancer Initiation and Progression

Joy Ware
(804) 828-9746
jlware@vcu.edu
http://www.pathology.vcu.edu/research/funcgeno/ware.html
Functional genomics, cancer

Colleen Jackson-Cook
804 828 9632 ext 118
jacksonc@hsc.vcu.edu
http://www.vipbg.vcu.edu/hg/dr/CJACKSON.shtml
Cancer, aneuploidy, Down's syndrome, chromosomal abnormalities

University of Iowa

John Menninger
john-menninger@uiowa.edu
http://www.biology.uiowa.edu/facultypage.php?ID=69
Accuracy of Protein Synthesis-Ribosome Editing - Cellular Aging

Bill Ballard
bill-ballard@uiowa.edu
http://www.biology.uiowa.edu/facultypage.php?ID=429
http://www.myweb.uiowa.edu/bballard/
Molecular Evolution, Comparative Genomics and Aging

Chi-Lien Cheng
chi-lien-cheng@uiowa.edu
http://www.biology.uiowa.edu/facultypage.php?ID=15
Molecular and Genetic Analysis of Leaf Greening and Senescence

University of Colorado at Boulder

Gretchen Stein
http://mcdb.colorado.edu/website_faculty/stein.gif
Control of cell proliferation in human cells (fibroblasts); cellular aging

Kenneth Krauter
http://mcdb.colorado.edu/website_faculty/krauter.gif
Human genetics; comparative DNA sequence analysis; behavioral genetics; complex trait mapping

Robert Poyton
http://mcdb.colorado.edu/website_faculty/poyton.gif
http://www.colorado.edu/MCDB/poytonlab/research.htm
Oxygen sensing and control of gene expression; mitochondrial biogenesis in the yeast Saccharomyces cerevisiae; oxidative stress and aging

Wake Forest University

Steven Akman
(336) 716-0231
http://www.wfubmc.edu/molecular_genetics/faculty/akman_steven.htm
Oxidative DNA damage and mutagenesis.

Donald Bowden
http://www.wfubmc.edu/molecular_genetics/faculty/bowden_donald.htm
Analysis of human diseases using DNA polymorphisms; emphasis on Type II diabetes, endocrine neoplasias, and chromosome structure.

Purdue University

Alan Friedman
765-494-5911
afried@purdue.edu
http://www.bio.purdue.edu/about/faculty/friedman/index.html
molecules and processes involved in infection, immunity, aging and repair

Kevin Hannon
765-494-5949
kmh@vet.purdue.edu
http://www.gradschool.purdue.edu/PULSe/faculty/hannon.html
Skeletal muscle atrophy

Sophie Lelievre
765-496-7793
sal@vet.purdue.edu
http://www.gradschool.purdue.edu/PULSe/faculty/lelievre.html
mechanisms by which the organization of components of the cell nucleus directs the expression and stability of the genome

University of Colorado Health Sciences Center

Jerome Schaack - Molec Bio
jerry.schaack@uchsc.edu
http://www.uchsc.edu/molbio/jschaack.html
development of vectors (adenovirus) for gene therapy

Mair E. Churchhill (F) - Molec Bio
Mair.Churchill@uchsc.edu
http://www.uchsc.edu/molbio/mchurchill.html
Structural Analysis of Gene-Regulatory Protein Complexes, DNA Modification, Quorum Sensing

Mingxia Huang
mingxia.huang@uchsc.edu
http://www.uchsc.edu/molbio/mhuang.html
cellular responses to DNA damage and envirionmental stress by genetic, biochem, and functional genomic approaches; yeast and human cells

Jessica Tyler
jessica.tyler@uchsc.edu
http://www.uchsc.edu/molbio/jtyler.html
chromatin assembly and remodeling

University of Virginia at Charlottesville

Anindya Dutta
ad8q@virginia.edu
http://www.healthsystem.virginia.edu/internet/bims_bmbg/cellcycle.cfm?uva_id=ad8q&hideintro=1
DNA Replication and Genomic Instability in Cancer Cells

William Pearson
wrp@virginia.edu
http://www.healthsystem.virginia.edu/internet/cancer-training/faculty.cfm?uva_id=wrp&hideintro=1
Glutathione S-Transferases; Protein Evolution; Computational Biology

North Carolina State University

Jill Barnes
(919) 513-6372
ja_barnes@unity.ncsu.edu
http://www2.ncsu.edu/unity/lockers/project/cvmaprhome/barnes_jill.htm
role of stress proteins (heat shock proteins) in cytoprotection and carcinogenesis

University of Wisconsin at Madison

Lynn Allen-Hoffman
http://www.cmb.wisc.edu/profiles/Allen_HoffmannLynn.html
NIKS immortal cell line

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